

Suppl. Table 1. Overview of protists for which transformation systems have been developed prior to the EMS initiative.

Phylum	Species	Transformation method	Construct (promoter)	Selectable marker; selecting agent	Reporter	Reference
Alveolates	<i>Symbiodinium microadriaticum</i>	Si carbide whiskers (SiCaW)	pMT Npt/GUS (nos, CaMV35S); pMT Hpt/GUS (<i>Agrobacterium</i> [Agro] p1'2')	<i>nptII</i> , <i>hpt</i> ; kanamycin, hygromycin*, G418	Beta-glucuronidase (GUS)	ten Lohuis & Miller (1998)
	<i>Symbiodinium</i> spp.	Agitation with Glass beads	<i>pCB302-gfp-AtRACK1C</i> , <i>pCambia-FABD2-gfp</i> (CaMV35S)	<i>bar</i> , <i>hpt</i> ; Basta, hygromycin	Green fluorescent protein (GFP)	Ortiz-Matamoros et al. (2015a)
		Glass beads and co-incubation with Agro	<i>pCB302-gfp-AtRACK1C</i> , <i>pCB302-gfp-MBD</i> , <i>pCB302-gfp-FABD2</i> (CaMV35S)	<i>bar</i> ; Basta	Green fluorescent protein (GFP)	Ortiz-Matamoros et al. (2015b)
	<i>Amphidinium carterae</i>	SiCaW	pMT Npt/GUS (nos, CaMV35S); pMT Hpt/GUS (<i>Agro</i> p1'2')	<i>nptII</i> , <i>hpt</i> ; hygromycin*, G418, kanamycin	GUS	ten Lohuis & Miller (1998)
Stramenopiles	<i>Phaeodactylum tricornutum</i>	Biolistics	(<i>fcp</i> [fucoxanthin chlorophyll-a or -c binding protein])	<i>ShBle</i> (phleomycin/zeocin)	Luciferase (LUC)	Falciatore et al. (1999)
		Biolistics	<i>pfcpA</i> (<i>fcp</i> [fucoxanthin chlorophyll-a or -c binding protein])	<i>ShBle</i> (phleomycin/zeocin)	Chloramphenicol acetyltransferase (CAT)	Apt et al. (1996)
		Electroporation	<i>pHY11-cat</i> (native NR promoter)	<i>cat</i> ; chloramphenicol	CAT	Niu et al. (2012)
		Conjugation	<i>p0521S/CEN6-ARSH4-HIS3/pfcpA</i>	<i>shble</i>	Cyan fluorescence protein (CFP), GFP, Yellow fluorescence protein (YFP)	Karas et al. (2015)
	<i>Cyclotella cryptica</i>	Biolistics	plasmids with <i>nptII</i> (diatom acetyl-CoA carboxylase promoter)	<i>nptII</i> ; G418	Expression of NPTII	Dunahay et al. (1995)

<i>Navicular saprophila</i>	Biolistics	plasmids with nptII (diatom acetyl-CoA carboxylase promoter)	nptII; G419	Expression of NPTII	Dunahay et al. (1995)
<i>Cylindrotheca fusiformis</i>	Biolistics	pHUPtag- <i>fcp</i> (fucoxanthin chlorophyll-a or -c binding protein)	<i>ble/frue</i> , <i>ble</i> /HUPtag; zeocin, kanamycin, hygromycin B		Fischer et al. (1999)
<i>Conticribra (Thalassiosira) weissflogii</i>	Biolistics	(<i>fcp</i> [fucoxanthin chlorophyll-a or -c binding protein])		Luciferase (LUC)	Falciatore et al. (1999)
<i>Pseudo-nitzschia multistriata</i> , <i>P. arenysensis</i>	Bioballistic	Histone H4	<i>Shble</i> , zeocin	Growth on selective medium	Sabatino et al. 2015
<i>Thalassiosira pseudonana</i>	Biolistics	pTpNR/GFP (nitrate inducible NR promoter)	<i>Nat</i> (nourseothricin)	EGFP	Poulsen & Chesley (2006)
<i>Thalassiosira pseudonana</i>	Conjugation	pTpExpPEPCK-YFP (LHCF9 promoter_	<i>Nat</i> (nourseothricin)	Yellow fluorescent protein (YFP)	Karas et al. (2015)
<i>Nannochloropsis</i> sp.	Electroporation	<i>pVCP1</i>	<i>shble</i>		Kilian et al. (2011)
<i>Nannochloropsis gaditana</i>	Electroporation	<i>pTUB/pHSP/pUEP/</i>	<i>shble</i>		Radakovits et al. (2013)
<i>Nannochloropsis oceanica</i>	Electroporation	<i>pCMV-EM7</i>	<i>sheble</i>		Osorio et al. (2019)
Archaeplastids <i>Chlamydomonas reinhardtii</i>	Glass beads	<i>pMN24</i> (containing native fragments)	<i>nr</i> ; nitrate	Growth on selective medium (nitrate)	Kindle (1990)
	Electroporation	<i>pJD67</i> carrying ARG7	<i>arg7</i>	Growth on corn starch	Shimogawara et al. (1998)
	Si carbide whiskers	<i>pMN24</i> based on pUC19 (containing native NR)	<i>nr</i> , nitrate	Growth on selective medium (nitrate)	Dunahay (1993)
	Biolistics	<i>pU12.6</i> (containing ASL)	argininosuccinate lyase (ASL); AS	Growth on selective medium	Debuchy et al. (1989)

	Biolistics	pUC19	OEEl, oxygen-evolving enhancer protein 1	Growth on selective medium (-acetate)	Mayfield & Kindle (1990)
	<i>Agrobacterium tumefaciens</i> **	T-DNA	<i>hpt</i> ; hygromycin	GUS, GFP	Kumar et al. (2004)
<i>Chlorella ellipsoidea</i>	Biolistics	pDO432		LUC	Jarvis & Brown (1991)
	Electroporation	(Ubil-Ω)		GUS	Chen et al. (2001)
<i>Chlorella saccharophila</i>	Electroporation	PBI221 (CaMVS35)		GUS	Maruyama et al. (1994)
<i>Chlorella vulgaris</i>	Protoplast transformation		<i>kanr</i> ; G418	Expression of human growth hormone (hGH)	Hawkins & Nakamura (1999)
	Electroporation	pMD18-Ap <i>cat</i> (NR promoter)	<i>cat</i> ; chloramphenicol		Niu et al. (2011)
<i>Haematococcus pluvialis</i>	Biolistics	pSV40-LacZ (SV40)		β-galactosidase (LacZ)	Teng et al. (2002)
<i>Ostreococcus tauri</i>	Electroporation	<i>Potluc</i> <i>Potox</i>	KanMX, G418 Nat1, cloNat	LUC	Corellou et al. [2009]
	Electroporation	PCR product including 1kbp of ferritin homologous sequence	KanMx, G418	LUC (Knock in)	Lozano et al. [2014]
<i>Dunaliella salina</i>	Electroporation	PBI221, pUGUS, pUΩGUS, P35UΩGUS (CaMVS35, Ubil-Ω)		GUS	Geng et al. (2003)
	Electroporation		<i>ble</i> ; Zeocin		Sun et al. (2005)***
	Biolistics	(CaMV35S promoter)	<i>bar</i> ; Basta	GUS	Tan et al. (2005)
	Biolistics	pDM307 (native CA [carbonic anhydrase] promoter)	<i>bar</i> ; Basta	Nitric oxide synthase (NOS)	Lü et al. (2005)

*found to be most effective; ** shown to work better than glass beads; *** some introduced DNA stayed as episomal plasmid DNA

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Suppl. Table 2. Physiological and ecological properties of protists selected for this study.

Species	Lifestyle (auto/hetero/mixo)	Axenic culture	Habitat	Cell wall	Cell structure	Life cycle	Planktonic
Archaeplastids							
<i>Ostreococcus lucimarinus</i>	photoautotroph	no	marine	no visible cell wall, just thin membrane	small (~1 µm); atypical prasinophyte (no flagella, no scales)	asexual by binary fission, possible sexual cycle	yes
<i>Bathycoccus prasinos</i>	photoautotroph	no	marine	organic scales	small (~2-3 µm); cells covered by scales of unknown organic material. no flagellum.	asexual by binary fission, possible sexual cycle	yes
<i>Micromonas commoda</i>	photoautotroph	yes (Rendered axenic using antibiotics for Worden et al. Science 2009. Tested here at start and end of each experiment using DAPI and test medium.)	marine	no visible cell wall, just thin membrane	small (~1-2 µm); single flagellum, no scales; phototactic (positive)	asexual by binary fission, possible sexual cycle	yes
<i>Micromonas pusilla</i>	photoautotroph	yes (Rendered axenic using antibiotics for Worden et al. Science 2009. Tested here at start and end of each experiment using DAPI and test medium.)	marine	no visible cell wall, just thin membrane	small (~1-2 µm); single flagellum; phototactic (positive); Encodes most of peptidoglycan pathway from cyanobacterial endosymbiont that formed plastid	asexual by binary fission, possible sexual cycle	yes
<i>Tetraselmis striata</i>	photoautotroph	no	marine coastal; most free-living but some animal symbionts	complex polysaccharide scales fuse to form a wall; cell anchored to wall by microtubules in four places	3-25 µm, two pairs of flagella with complex scales etc covering, one large chloroplast; accumulates HUFAs	flagellated stage, vegetative non-motile stage (usually dominant), cyst	yes
<i>Pyramimonas parkeae</i>	photoautotroph	not tested	marine	wall-less	20 x 15 µm	four flagella	yes
Haptophytans							
<i>Isochrysis galbana</i>	photoautotrophic	no	marine	thin layer of organic scales, non-calcified	5-6µm, two flagella, 2 chloroplasts, stigma, oil droplets	Asexual by binary fission, cyst	yes

<i>Emiliana huxleyi</i>	photoautotrophic	no	marine, broadly distributed	calcium carbonate plates (coccoliths)	~5 µm, no flagella	diploid calcified stage, motile non-calcified haploid stage	yes
Rhizarians							
<i>Amorphochlora (Lotharella) amoebiformis</i>	photomixotrophic (eat bacteria)	no	marine	none	Amoeboid cell (8-15 µm) with many filopodia.	asexual by binary fission	no
<i>Bigelowiella natans</i>	photomixotrophic (eat bacteria)	not tested	marine, broadly distributed	vegetative cells usually naked; this cell wall only in 'cysts' in old cultures	Amoeboid, thin filopodia (thread-like pseudopodia; sometimes reticulopodia); or coccoid cells with multilayered cell wall; or unflagellated zoospores. store b-1,3 glucan (not starch). Mitochondria with tubular cristae. Nucleomorph genome ~400 kb (3 linear chromosomes), coding 17 plastid genes and ~350 housekeeping genes with lots of small (~20 bp) introns. Secondary endosymbiosis between cercozoan host and green algal symbiont	some species have all three cell types, many are missing one or another; which form is the main vegetative stage differs among species. Chlorarachnion may have amoeboid gamete!	yes
Stramenopiles							
<i>Fragilariopsis cylindrus</i>	photoautotrophic	not tested	polar oceans and sea ice; ice edge blooms	silica	~4 µm, pennate		yes
<i>Thalassiosira pseudonana</i>	photoautotrophic	yes (Bacterial growth checked in LB plates)	oceanic, coastal temperate, possible bacterial symbiont	silica and organics (long-chain polyamines, chitin, proteins)	~5-10 µm	size reduction–restitution cycle (SRRC): asexual by binary fission accompanied by cell size reduction, cell size restored in a sexual cycle	yes
<i>Seminavis robusta</i>	photoautotrophic	not tested, but maintained with a cocktail of Penicillin, Ampicillin, Gentamycin, and Streptomycin	benthic	silica	~10x50 µm; pennate	Size reduction-restitution cycle; sexual cycle with two known mating types	no
<i>Pseudo-nitzschia multiseries</i>	photoautotrophic	no	HAB-forming; common during coastal upwelling; significant	silica	~5x100 µm; pennate, chain-forming	asexual by binary fission accompanied by cell size reduction, cell	yes

			player in local food web; can be toxic; neretic to open ocean			size restored in a sexual cycle	
<i>Heterosigma akashiwo</i>	photoautotrophic	no	diverse; some form HAB	naked	~50-100 µm, heterokont flagella		yes
<i>Aurantiochytrium limacinum</i>	osmoheterotrophic; stores PUFAS	yes (checked by 16S PCR, by microscopy, also, neither genomic nor transcriptomic sequencing have suggested the presence of any other organism)	marine (plant detritus)	sulfated polysaccharide scales	4 - 20 µm; grow attached by ectoplasmic net elements or in suspension	probably asexual reproduction by zoospores	mero ¹
<i>Caecitellus sp.</i>	phagoheterotrophic	not tested		none?	<5 µm; gliding motility, raptorial feeding		yes
<i>Nannochloropsis oceanica</i>	photoautotroph	not tested (bought as axenic, but not retested)	marine waters worldwide	Smooth cell wall, composed of principally cellulose and algaenan, with papilla	<5 µm ovoid cells, non-motile	asexual reproduction; may be ameiotic	yes
<i>Phaeodactylum tricornutum</i>	photoautotrophic	yes (Provasoli antibiotic treatment, DAPI staining)	brackish and marine waters worldwide	oval morphotype silicified, other morphotypes very lightly silicified, sulfated a-mannan decorated with glucuronic residues	polymorphic (exists as oval, fusiform, triradiate, cruciform morphotype), ~5x30 µm (fusiform), ~10 µm ovoid gliding motility (oval), can form chains	asexual by binary fission, possible sexual cycle	yes (oval type benthic)
Alveolates							
<i>Euplotes crassus</i>	phagoheterotrophic	no	marine	rigid pellicle	~50-100 µm; hypotrichous ciliate	asexual by binary fission and sexual by conjugation or autogamy	yes
<i>Euplotes focardi</i>	phagoheterotrophic	no	marine f=antarctic	rigid pellicle	~50-100 µm; hypotrichous ciliate	asexual by binary fission and sexual by conjugation	yes
<i>Chromera velia</i>	photoautotroph?	not tested	coral reef coral-associated, possibly non-facultative symbiont	thick cell wall	7.0 × 7.6 µm, coccoid stage with no flagella	sex not observed, forms flagellate stages	mero ¹
<i>Perkinsus marinus</i>	osmoheterotroph, obligate parasite	yes (The ATCC repository sells it as axenic)	parasite of marine molluscs	Yes	2-10 µm; suggested apicoplast	direct cycle, zoospores and	Both as trophozoite and

						trophozoites, sex not observed	flagellated stage
<i>Oxyrrhis marina</i>	heterotrophic (phagotrophic; omnivorous)	no	plankton; global coastally except polar; can form blooms	naked (no theca) except has scales	20-30 µm		yes
<i>Hematodinium</i> sp.	parasite	yes	crustacean hemolymph	naked at trophont stage	Cultured as asexual multinucleated trophonts (up to 50 µm)	Complex asexual stages, non-flagellate; mononucleated sexual zoospores	Only at zoospore stage
<i>Fugacium</i> (<i>Symbiodinium</i>) <i>kawagutii</i>	photoautotrophic symbionts (maybe some phagotrophy?)	no (Grown under 100 µg/ml Ampicillin, 50 µg /ml Kanamycin and 50 µg/ml Streptomycin)	intra- or intercellular symbionts of marine invertebrates	naked swimming cell but cellulose wall in nonmotile phase	~10 µm	motile (free-swimming) and non-motile (in host) phases; only the latter grows and divides	
<i>Alexandrium catenella</i>	photoautotroph	no (Grown under 100 µg/ml Ampicillin, 50 µg /ml Kanamycin and 50 µg/ml Streptomycin)	HAB-forming phytoplankton; produce saxitoxin (PSP)	cellulose plates	~25 µm, usually chains of 2, 4, 8	asexual reproduction by binary fission; sexual reproduction to form a cyst	yes
<i>Breviolum</i> (<i>Symbiodinium</i>) sp.	photoautotrophic symbionts (maybe some phagotrophy?)	not tested	intra- or intercellular symbionts of marine invertebrates	naked swimming cell but cellulose wall in nonmotile phase	~10 µm	motile (free-swimming) and non-motile (in host) phases; only the latter grows and divides	
<i>Crypthecodinium cohnii</i>	heterotrophic (glucose, acetate, propionic acid); may predate via peduncle	yes (The NCMA repository sells it as axenic; the samples are routinely tested for bacterial growth in marine liquid bacterial medium)	brackish, littoral, neritic; often around macrophytes like Fucus. temperate and tropical	very thin cellulose	~15-20 µm; produce carotenoids in the light	stores starch during log phase, DHA mainly in cysts?; swimming cells and cysts.	
<i>Amphidinium carterae</i>	photoautotroph	no (wild-type is not axenic, but can be grown axenically)	HAB-forming phytoplankton	naked?	~25 µm, solitary	Asexual by binary fission	yes
<i>Karlodinium veneficum</i>	mixotroph (predatory)	no	HAB-forming, toxic, marine, planktonic	naked	~10 µm		yes

(Grown under 400
µg/ml Ampicillin)

Discobans

<i>Bodo saltans</i>	phagoheterotrophic	no (the cultures which carried bacterial populations from the original isolation were inoculated with bacteria (Klebsiella pseudomonas) as a food source	broadly distributed	none?	<i>B. saltans</i> is 4-5 µm with two flagellae		no? <i>B. saltans</i> attaches to surfaces by tip of long (posterior) flagellum
<i>Diplonema papillatum</i>	phagoheterotrophic	yes (checked by microscopy, 100µg/ml of chloramphenicol added in the media)	free-living planktonic?	no pellica	~16 µm, two flagella of equal length, two subapical openings		yes
<i>Eutreptiella gymnastica</i>	photoautotrophic	not tested	neritic, cosmopolitan	flexible pellicle	15-20 µm, two flagella, reddish eyespot, paramylon granules in cytoplasm, vigorous metabolism often observed	can form a cyst with layered cell wall	yes
<i>Naegleria gruberi</i>	phagoheterotrophic	yes (Axenic culture was tested microscopically along with DAPI staining).	wet soil and freshwater	naked	amoeboid flagellate; the amoeba lacks microtubule cytoskeleton, flagellate has elaborate one including the flagella; de novo synthesis of basal body during transformation from former to latter	apparently the genome revealed two distinct haplotypes	no

Opisthokonts

<i>Pirum gemmata</i>	osmoheterotroph; stores glycogen	yes (Sold axenic by ATCC)	peanut worm (<i>Phascolosoma agassizii</i>) gut contents, BC, 2004	composition unknown but fibrous and woven; contain membrane-bound tubular extensions of the cytoplasm with tubules.	vegetative cells ~50-150 µm; large central vacuole with cytoplasm mostly pressed against periphery; multinucleate with nuclei 2 to 4 µm; sporulation happens in half an hour; endospores ~5 µm and 'weakly amoeboid'	walled cells divide internally to produce lots of endospores, which are released through parental cell wall in spurts	no
<i>Sphaeroforma arctica</i>	phagoheterotrophic	yes (axenic growth checked in agar plates)	invertebrate symbiont; isolated from Gammarus; arctic	carbohydrate, mostly N-acetyl-glucosamine (chitin?)	simple round cells, lots of DHA and EPA in cell membranes but not accumulated to high levels in lipid bodies	very simple growth from 5-7 µm cells for 48 hr to 35-40 µm, then	no

						releasing ~120 new cells	
<i>Abeoforma whisleri</i>	osmoheterotroph; stores glycogen	yes (axenic growth checked in agar plates)	mussel (<i>Mytilus</i> sp.) gut contents, BC, 2007	have both cell wall and extracellular matrix; wall sometimes very thick; composition unknown but fibrous and woven; contain membrane-bound tubular extensions of the cytoplasm with tubules.	vegetative cells mostly spherical ~50 µm but some plasmodial; endospores, plasmodia, and hyphae-like structures observed; say dispersal amoebae 'function in post reproductive dispersal' and are uninucleate	walled spherical cells, plasmodia, amoebae; asexual reproduction by dispersal amoebae, endospores, binary fission and budding.	no
<i>Salpingoeca rosetta</i>	phagoheterotrophic	no	free-living planktonic and benthic thecate	a proteinaceous and polysaccharide matrix	cell body 3-10 µm; single apical flagellum surrounded by a collar of 30-40 actin-filled microvilli	asexual by longitudinal fission; sexual reproduction triggered by nutrient deprivation and a secreted chondroitin lyase from <i>Vibrio</i> bacteria; dynamic life history includes unicellular and multicellular forms	yes

¹ Meroplanktonic organisms spend only a portion of their lives as plankton.

Suppl. Table 4. Transformation methods applied in this study.

A. Electroporation, B. Biolistics, C. Microinjection, D. Chemical transformation, E. Conjugation and F. Glass bead abrasion conditions.

A. Electroporation

Species	Electroporation devices (type of transformation)	Used program/setting	Survival rate
Archaeplastids			
<i>Ostreococcus lucimarinus</i>	Gene Pulser Xcell	1200V, 25μF	30-50%
<i>Bathycoccus prasinos</i>	Gene Pulser Xcell	1500V, 25μF	50%
<i>Micromonas commoda</i>	LONZA®	SF Buffer with pulse EH-100, EO-100, EN-138 and EW-113	10-11%
<i>Micromonas pusilla</i>	BioRad Gene Pulser	1000V, 10μF, 400Ω 800V, 25μF, 400Ω 600V, 50μF, 400Ω	n/a
<i>Pyramimonas parkeae</i>	Gene Pulser Xcell	1500V, 25μF	n/a
	Gene Pulser Xcell	300V, 500μF	n/a
	Gene Pulser Xcell	2500V, 25μF	n/a
	Gene Pulser Xcell	310V, 960μF	n/a
	Gene Pulser Xcell	420V, 960μF	n/a
	Gene Pulser Xcell	300V, 200μF	n/a
	Gene Pulser Xcell	100V, 100μF	n/a
	Gene Pulser Xcell	100V, 300μF	n/a
	Amaza Nucleofector II	preset program X-001	n/a
	Amaza Nucleofector II	preset program T-020	n/a
	Amaza Nucleofector II	preset program T-023	n/a
	Amaza Nucleofector II	preset program U-035	n/a
Rhizarians (Chlorarachniophytes)			
<i>Amorphochlora (Lotharella) amoebiformis</i>	Gene Pulser Xcell	120 V, 25 ms square wave, 0.2 cm cuvette	20-30%
<i>Bigelowiella natans</i>	Amaza Nucleofector II	preset program X-001	n/a
	Amaza Nucleofector II	preset program T-020	n/a
	Amaza Nucleofector II	preset program T-023	n/a
	Amaza Nucleofector II	preset program U-035	n/a
	Gene Pulser Xcell	7× poring pulse: 300V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
	Gene Pulser Xcell	7× poring pulse: 250V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
	Gene Pulser Xcell	7× poring pulse: 350V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
Stramenopiles (Diatoms, Bacillariophytes, Raphidophytes)			

<i>Seminavis robusta</i>	BTX ECM 2001	Single pulse conditions: 5 V AC for 5 sec, 300 V pulse for 5, 2.5, 0.5, or 0.1 msec. 5 pulse conditions: 5 V AC for 5 sec, 300 V pulse for 5, 2, 1, or 0.1 msec 10 pulse conditions: 5 V AC for 5 sec, 300 or 500 V pulse for 0.1 or 0.05 msec (only for 500 V) Poring pulse: 150, 200, 225, 250, 275, or 300 V, 10% decay rate; 5 ms length, 50 ms interval Transfer pulse: 8 V, 40% decay rate, 50 ms length, 50 ms interval, alternating polarity	n/a
	NEPA21		n/a
<i>Heterosigma akashiwo</i>	Gene Pulser Xcell	50/75 V, 25 μ F and $\infty\Omega$	80%
<i>Aurantiochytrium limacinum</i>	Gene Pulser (BIO-RAD)	2 pulses: 450V, 25 μ F, 1000 Ω (~5 ms)	2.64%
	NEPA	2 pulses: 250 V, 4 ms	2.48%
	NEPA	2 pulses: 275 V, 8 ms	4.13%
	NEPA	2 pulses: 300 V, 12 ms	7.27%
<i>Nannochloropsis oceanica</i>	Gene Pulser II	1800V, 50 μ F, 20 ms	n/a
	Gene Pulser II	1000V, 50 μ F, 20 ms	n/a
Alveolates			
<i>Euplotes crassus</i>	BioRad Gene Pulser	200V, 25 μ F, 100 Ω	50-60%
<i>Chromera velia</i>	Amaza Nucleofector II	preset programs X-001	n/a
	Amaza Nucleofector II	preset program T-020	n/a
	Amaza Nucleofector II	preset program T-023	n/a
	Gene Pulser Xcell	1500V, 25 μ F	n/a
	Gene Pulser Xcell	300V, 500 μ F	n/a
	Gene Pulser Xcell	2500V, 25 μ F	n/a
	Gene Pulser Xcell	310V, 960 μ F	n/a
<i>Perkinsus marinus</i>	Amaza Nucleofector II	preset program D-023	n/a
<i>Oxyrrhis marina</i>	BioRad's MicroPulser	DIC	n/a
	BioRad's MicroPulser	DIC -2 shocks	n/a
	BioRad's MicroPulser	SHS	n/a
	BioRad's MicroPulser	SC2	n/a
<i>Hematodinium sp.</i>	Amaza Nucleofector II	D-023 and X-001	<1%
<i>Fugacium kawagutii</i>	BioRad's MicroPulser	DIC	n/a
	BioRad's MicroPulser	DIC -2 shocks	n/a
	BioRad's MicroPulser	SHS	n/a

	BioRad's MicroPulser	SC2	n/a
<i>Alexandrium catenella</i>	BioRad's MicroPulser	DIC	n/a
	BioRad's MicroPulser	DIC -2 shocks	n/a
	BioRad's MicroPulser	SHS	n/a
	BioRad's MicroPulser	SC2	n/a
<i>Breviolum (Symbiodinium) sp.</i>	NePA21 Electro-kinetic transfection system	Poring pulse: 300V, 10ms Transfer pulse: 8V, 50ms	n/a
<i>Crypthecodinium cohnii</i>	Amaza Nucleofector II	preset program D-023	79%
	Amaza Nucleofector II	preset program A-020	85%
	Amaza Nucleofector II	preset program T-020	67%
	Amaza Nucleofector II	preset program T-030	53%
	Amaza Nucleofector II	preset program X-001	62%
	Amaza Nucleofector II	preset program X-001	71%
	Amaza Nucleofector II	preset program L-029	77%
	Amaza Nucleofector II	preset program X-003	n/a
	Amaza Nucleofector II	preset program X-005	n/a
	Amaza Nucleofector II	preset program X-033	n/a
	Amaza Nucleofector II	preset program Y-003	n/a
	Amaza Nucleofector II	preset program Y-005	n/a
	Amaza Nucleofector II	preset program Y-033	n/a
	Amaza Nucleofector II	preset program Z-007	n/a
	Amaza Nucleofector II	preset program Z-023	n/a
	Amaza Nucleofector II	preset program Z032	n/a
	Amaza Nucleofector II	preset program BAC1	n/a
	Amaza Nucleofector II	preset program BAC2	n/a
	Amaza Nucleofector II	preset program BAC3	n/a
	Amaza Nucleofector II	preset program BAC4	n/a
	Amaza Nucleofector II	preset program BAC5	n/a
	Amaza Nucleofector II	preset program BAC6	n/a
	Amaza Nucleofector II	preset program BAC7	n/a
	Amaza Nucleofector II	preset program L-029	n/a
	Amaza Nucleofector II	preset program L-029	n/a
	Microfluidics	Straight 313 V	n/a
	Microfluidics	Straight 625 V	n/a
	Microfluidics	Straight 938 V	n/a
	Microfluidics	Straight 1250V	n/a
	Microfluidics	Straight 1563 V	n/a
	Microfluidics	Divergent 333 V	n/a
	Microfluidics	Divergent 667 V	n/a
	Microfluidics	Divergent 1000 V	n/a
	Microfluidics	Divergent 1333 V	n/a
	Microfluidics	Divergent 1667 V	n/a

	Microfluidics	Divergent 5000 V	n/a
	Lipofectamine		90-100%
<i>Amphidinium carterae</i>	Amaxa Nucleofector 4D	X-100, D-023, L-029 and EH 100.	n/a
	NEPA electroporator	Poring pulse: 150V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 200V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 225V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 250V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 275V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 300V, length: 5 ms, interval 50 ms, number 1 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 300V, length: 5 ms, interval 50 ms, number 4 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 300V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 300V, length: 5 ms, interval 50 ms, number 9 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
<i>Karlodinium veneticum</i>	BioRad's MicroPulser	DIC	n/a
	BioRad's MicroPulser	DIC -2 shocks	n/a
	BioRad's MicroPulser	SHS	n/a
	BioRad's MicroPulser	SC2	n/a
Discobans (Euglenozoans and Heteroloboseans)			
<i>Bodo saltans</i>	NePA21 Electro-kinetic transfection system	Poring pulse: 250V, 25ms Transfer pulse: 60V, 99ms	30-50%
<i>Diplonema papillatum</i>	BTX	1600V, 25W, 50mF	10 %
	Amaxa Nucleofector II	preset program X-001	80-90 %
	Amaxa Nucleofector II	preset program X-014	40-50 %

<i>Eutreptiella gymnastica</i>	Gene Pulser Xcell	1500V, 25μF	n/a
	Gene Pulser Xcell	300V, 500μF	n/a
	Gene Pulser Xcell	2500V, 25μF	n/a
	Gene Pulser Xcell	310V, 960μF	n/a
	Gene Pulser Xcell	420V, 960μF	n/a
	Gene Pulser Xcell	300V, 200μF	n/a
	Gene Pulser Xcell	100V, 100μF	n/a
	Gene Pulser Xcell	100V, 300μF	n/a
	Gene Pulser Xcell	200V, 100μF	n/a
	Gene Pulser Xcell	200V, 300μF	n/a
	Gene Pulser Xcell	350V, 1000μF	n/a
	Gene Pulser Xcell	7× poring pulse: 300V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
	Gene Pulser Xcell	7× poring pulse: 250V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
	Amaza Nucleofector II	preset program X-001	n/a
	Amaza Nucleofector II	preset program T-020	n/a
	Amaza Nucleofector II	preset program T-023	n/a
<i>Naegleria gruberi</i>	BioRad Gene Pulser xCell	175V, 500μF, 400Ω	10-20 %
	Amaza Nucleofector II	preset program X-29	40-50%
Opisthokonts			
<i>Sphaeroforma arctica</i>	Neon	1000-2500V, 10-40 ms, 1-3 pulses	n/a (not successful)
	Lipofectamina		n/a (not successful)
	LONZA®	16 preset codes P3/P4/P5 buffer	n/a (not successful)
<i>Abeoforma whisleri</i>	Neon (invitrogen)	1300V, 25ms, pulse	60%
	LONZA®	preset program EN-138 P3 buffer	70%
	CaCl+Glycerol		n/a (not successful)
	Lipofectamine		100% (not successful)
<i>Salpingoeca rosetta</i>	LONZA®	SF Buffer with pulse CM156	50%

B. Biolistics

Species	Biolistics device	Settings	Survival rate
Archaeplastids			
<i>Tetraselmis striata</i>	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	0.6μm AuNPs, rupture disc 1550 or 2000 psi, 6cm gap	n/a
<i>Pyramimonas parkeae</i>	PDS-1000/He	0.6 or 1μm AuNPs, rupture disc 1350 psi, 6cm gap	n/a

Haptophytes			
<i>Isochrysis galbana</i>	PDS-1000/He	0.7µm Tungsten beads rupture disc 1350 psi, 6cm gap	n/a
Rhizarians (Chlorarachniophytes)			
<i>Amorphochlora (Lotharella) amoebiformis</i>	PDS-1000/He	1 µm AuNPs, rupture disc 450 psi, 4cm gap	n/a
<i>Bigelowiella natans</i>	PDS-1000/He	0.6µm AuNPs, rupture disc 1350 psi, 6cm gap	n/a
Stramenopiles (Diatoms, Bacillariophytes, Raphidophytes)			
<i>Fragilariopsis cylindrus</i>	PDS-1000/He	0.7µm Tungsten beads, rupture disc 1550 psi, 6cm gap	n/a
<i>Seminavis robusta</i>	PDS-1000/He	0.55 µm AuNPs or 1.1 µm WNPs, 1550 psi, 3 µg/mL DNA non-linearized and linearized (but not CIP-treated), at 3, 6, 9, and 12 cm gap distances	n/a
Alveolates			
<i>Euplotes crassus</i>	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	0.6 µm or 1.6 µm AuNPs, rupture disc 1550 psi, helium pressure 1750 psi, vacuum 26 inches Hg, gap distance 3/8 inches, in 10 mM HEPES pH 7.4	80-90%
<i>Chromera velia</i>	PDS-1000/He	0.6µm AuNPs, rupture disc 1350 psi, 6cm gap	n/a
<i>Hematodinium</i> sp.	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	rupture disc 1550 psi 550 nm diameter gold particles	10%
<i>Fugacium kawagutii</i> *	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	0.7 or 1.1 µm Tungsten; rupture disc 450, 650, 900, 1100, 1350, 1550 psi; vacuum 28 inches Hg; 7.5cm gap	n/a
<i>Alexandrium</i> * <i>catenella</i>	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	0.7 or 1.1 µm Tungsten; rupture disc 450, 650, 900, 1100, 1350, 1550 psi; vacuum 28 inches Hg; 7.5cm gap	n/a
<i>Cryptothecodinium cohnii</i>	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	rupture disc 1550 psi 550 nm diameter gold particles	70-80%
<i>Amphidium carterae</i>	Bio-Rad Biolistics PDS-1000/He	rupture disc 1550 psi 550 nm diameter gold particles	n.d.
Discobans (Euglenozoans and Heteroloboseans)			
<i>Eutreptiella gymnastica</i>	PDS-1000/He	0.6 or 1µm AuNPs, rupture disc 1350 psi, 6cm gap	n/a

*This part of work was assisted by Kaidian Zhang from Xiamen University, China.

C. Microinjection

Species	Microinjection device	Used setting	Survival rate
Alveolates			
<i>Euplotes crassus</i>	Eppendorf InjectMan NI 2	With Eppendorf Femtotips Microinjection Capillary Tip	2-10%

D. Chemical transformation

Species	Transfection reagent	Used setting	Survival rate
Archaeplastids			
<i>Pyramimonas parkeae</i>	Lipofectamine® 3000 Transfection Reagent (Invitrogen)	DNA-Lipofectamine complex prepared according to the supplier.	n/a
Rhizarians (Chlorarachniophytes)			
<i>Bigelowiella natans</i>	Lipofectamine® 3000 Transfection Reagent (Invitrogen)	DNA-Lipofectamine complex prepared according to the supplier.	n/a
Alveolates			
<i>Euplotes crassus</i>	Lipofectamine® 2000 or Lipofectamine® 3000 Transfection Reagent (Invitrogen)	DNA-Lipofectamine complex prepared according to the supplier.	100%
	Effectene Transfection Reagent (QIAGEN)	DNA-Effectene complex prepared according to the supplier with a double amount of DNA	10-20%
	FuGENE HD Transfection Reagent (Promega)	DNA-FuGENE complex prepared according to the supplier.	50-60%
Discobans (Euglenozoans and Heteroloboseans)			
<i>Eutreptiella gymnastica</i>	Lipofectamine® 3000 Transfection Reagent (Invitrogen)	DNA-Lipofectamine complex prepared according to the supplier.	n/a

E. Conjugation

Species	Coincugation (species co-incubated)	Survival rate	Efficiency
Stramenopiles (Diatoms, Bacillariophytes, Raphidophytes)			
<i>Thalassiosira pseudonana</i>	<i>E. coli</i> EPI300	n/a	~10%
<i>Heterosigma akashiwo</i>	Agrobacterium	10-15%	n/a
Alveolates			
<i>Oxyrrhis marina</i>	<i>E. coli</i>	100%	1-5%
<i>Karlodinium veneficum</i>	<i>E. coli</i>	100%	n/a

<i>Alexandrium catenella</i>	<i>E. coli</i>	100%	n/a
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F. Glass bead abrasion

Species	Co-incubation (species co-incubated)	Survival rate	Efficiency
Stramenopiles (Diatoms, Bacillariophytes, Raphidophytes)			
<i>Heterosigma akashiwo</i>	n/a	80%	n/a
Alveolates			
<i>Perkinsus marinus</i>	n/a	80-90%	0.01%-1%
<i>Hematodinium</i> sp.	n/a	40-50%	0%
<i>Amphidinium carterae</i>	None	No data	0%
	Polyethylene glycol	No data	0%

Suppl. Table 5: List of protists selected for this study including links to their transformation protocols (protocols.io) and vector sequences. For contacting particular laboratories, see Suppl. Table 6. For the vector sequences and maps, see Suppl. Notes 1.

Species	Source of organism/ Strain/Culture collection number	Principal Investigator (PI)	Other Investigators	protocols.io links (including construct maps and their sequences)	Sequences submitted (Accession No.) / published sequences
Archaeplastids					
<i>Ostreococcus lucimarinus</i>	RCC802	François-Yves Bouget	Jean-Claude Lozano Valérie Vergé	https://www.protocols.io/view/election-of-stable-transformants-in-ostreococcus-zj2f4qe https://www.protocols.io/view/transient-luciferase-expression-in-ostreococcus-ot-hcib2ue https://www.protocols.io/view/transient-transformation-of-ostreococcus-species-o-g86bzze http://dx.doi.org/10.17504/protocols.io.g86bzze	pHAPT:luc vector (Djouani-Tahri <i>et al.</i> , 2011) was used as a template for preparation of linear construct
<i>Bathycoccus prasinos</i>	RCC4222	François-Yves Bouget	Jean-Claude Lozano Valérie Vergé	https://www.protocols.io/view/election-of-stable-transformants-in-ostreococcus-zj2f4qe http://dx.doi.org/10.17504/protocols.io.hcib2ue http://dx.doi.org/10.17504/protocols.io.g86bzze	pHAPT:luc vector (Djouani-Tahri <i>et al.</i> , 2011) was used as a template for preparation of linear construct
<i>Micromonas commoda</i> ¹	CCMP 2709 (genome sequenced, axenic version of RCC299)	Alexandra Z. Worden	Manuel Ares Jian Guo Lisa Sudek	http://dx.doi.org/10.17504/protocols.io.8p9hvr6 http://dx.doi.org/10.17504/protocols.io.8p8hvrw	
<i>Micromonas pusilla</i>	CCMP 1545	François-Yves Bouget Alexandra Z. Worden	Manuel Ares Jian Guo Jean-Claude Lozano Lisa Sudek Valérie Vergé	https://www.protocols.io/view/plasmid-dnas-designed-for-expression-in-micromonas-i9wch7e	
<i>Tetraselmis striata</i>	KAS-836	Heriberto Cerutti Thomas Clemente	Patrick Beardslee Fulei Luan Xiaoxue Wen	http://dx.doi.org/10.17504/protocols.io.hjt4nn	GenBank (KY886895)
<i>Pyramimonas parkeae</i>	SCCAP K-0007	Vladimir Hampl	Natalia Ewa Janowicz Anna M.G. Novák Vanclová	https://www.protocols.io/view/protocols-for-mrna-electroporation-hh4b38w https://www.protocols.io/view/nucleofection-of-pyramimonas-parkeae-chromera-velibucanw https://www.protocols.io/view/biolistic-transformation-experiment-on-eutrepitiellibvcán6	

Haptophytes					
<i>Isochrysis galbana</i>	CCMP 1323	Colin Brownlee	Cecilia Balestreri Andrea Highfield Rowena Stern Glen Wheeler	https://www.protocols.io/view/biolistic-transformation-of-isochrysis-galbana-2pugdnw https://www.protocols.io/view/method-for-electroporation-of-isochrysis-galbana-c-hmab42e	GenBank (MK903009 - pigNAT construct) (MK903010 -PCR product of transgene)
<i>Emiliana huxleyi</i>	CCMP 1516	Colin Brownlee	Cecilia Balestreri Andrea Highfield Rowena Stern Glen Wheeler	http://dx.doi.org/10.17504/protocols.io.8tzhwp6	
Rhizarians					
<i>Amorphochlora (Lotharella) amoebiformis</i>	CCMP 2058	Yoshihisa Hirakawa	Kodai Fukuda	http://dx.doi.org/10.17504/protocols.io.35hgq36	
<i>Bigelowiella natans</i>	CCMP 2755	Vladimir Hampl	Natalia Ewa Janowicz Anna M.G. Novák Vanclová	https://www.protocols.io/view/protocols-for-mrna-electroporation-hh4b38w https://www.protocols.io/view/nucleofection-of-pyramimonas-parkeae-chromera-veli-ibucanw https://www.protocols.io/view/biolistic-transformation-experiment-on-eutreptiell-ibvcn6	
Stramenopiles					
<i>Fragilariopsis cylindrus</i>	CCMP 1102	Thomas Mock	Amanda Hopes	http://dx.doi.org/10.17504/protocols.io.z39f8r6 https://www.protocols.io/view/biolistic-transformation-of-polar-diatom-fragilari-z39f8r6	
<i>Thalassiosira pseudonana</i>	CCMP 1335	Christopher L. Dupont Pamela Silver	Jernej Turnsek	http://dx.doi.org/10.17504/protocols.io.jfncjme http://dx.doi.org/10.17504/protocols.io.nbzdap6 http://dx.doi.org/10.17504/protocols.io.7ghhj6	
<i>Seminavis robusta</i>	DCG 0498 (D6) DCG 0514 (VM3-4)	Aaron Turkewitz	Luke Noble Matthew Rockman Lev Tsy-pin	http://dx.doi.org/10.17504/protocols.io.4p8gvrr	
<i>Pseudo-nitzschia multiseries</i>	MLML-EBL culture collection, strain 15091C3 Unavailable due to culture collapse (after 3 years of cultivation), but DNA and RNA stocks are available	G. Jason Smith	Deborah Robertson April Woods	http://dx.doi.org/10.17504/protocols.io.7vhhn36	http://dx.doi.org/10.17504/protocols.io.7vhhn36

<i>Heterosigma akashiwo</i>	CCMP 2393	Kathryn Coyne	Pamela Green	http://dx.doi.org/10.17504/protocols.io.4qggvwtw http://dx.doi.org/10.17504/protocols.io.4qhgv6 https://www.protocols.io/view/modified-genomic-dna-extraction-method-for-heteros-himb4c6 https://www.protocols.io/view/modified-total-rna-extraction-for-heterosigma-akas-hipb4dn https://www.protocols.io/view/efforts-to-transform-heterosigma-akashiwo-using-an-4ytgxwn	
<i>Aurantiochytrium limacinum</i>	ATCC MYA-1381	Jackie Collier	Joshua Rest Mariana Rius	http://dx.doi.org/10.17504/protocols.io.8xyhxpww http://dx.doi.org/10.17504/protocols.io.hg6b3ze http://dx.doi.org/10.17504/protocols.io.pgtjdjwn	https://www.addgene.org/Jackie_Collier/ (for pUC19_GZG, pUC19_18GZG)
<i>Caecitellus</i> sp.	Unavailable due to culture collapse	Patrick Keeling	Elisabeth Hehenberger Nicholas A. T. Irwin	https://www.protocols.io/view/electroporation-of-caecitellus-sp-with-fitc-dextra-35kgq4w	
<i>Nannochloropsis oceanica</i>	CCMP 1779	Peter von Dassow	Fernan Federichi Isaac Nuñez Tamara Matute Albane Ruaud Jorge Ibañez	http://dx.doi.org/10.17504/protocols.io.7r8hm9w http://dx.doi.org/10.17504/protocols.io.h3nb8me	https://doi.org/10.5281/zenodo.3463694
<i>Phaeodactylum tricornutum</i>	CCAP1055/1	Andrew E. Allen	Mark Moosburner Chris Bowler	http://dx.doi.org/10.17504/protocols.io.4abgsan http://dx.doi.org/10.17504/protocols.io.4acgsaw http://dx.doi.org/10.17504/protocols.io.4bmgs6 http://dx.doi.org/10.17504/protocols.io.7gihjue	http://dx.doi.org/10.17504/protocols.io.7gnhjve
Alveolates					
<i>Euplotes crassus</i>	CCAP 1624/31	Cristina Miceli	Rachele Cesaroni Lawrence A. Klobutcher Mariusz Nowacki Angela Piersanti Sandra Pucciarelli Estienne Swart	https://www.protocols.io/view/euplotes-miceli-lab-2a8gahw/protocols	
<i>Euplotes focardii</i>	CCAP 1624/34	Cristina Miceli	Angela Piersanti Sandra Pucciarelli	https://www.protocols.io/view/euplotes-miceli-lab-2a8gahw/protocols	
<i>Chromera velia</i>	CCMP 2878	Vladimir Hampl	Natalia Ewa Janowicz Anna M.G. Novák Vanclová	https://www.protocols.io/view/protocols-for-mrna-electroporation-hh4b38w	

				https://www.protocols.io/view/nucleofection-of-pyramimonas-parkeae-chromera-veli-ibucanw https://www.protocols.io/view/biolistic-transformation-experiment-on-eutreptiell-ibvcn6	
<i>Perkinsus marinus</i> ²	ATCC PRA240	José A. Fernández Robledo Senjie Lin Ross Waller	Duncan B. Coles Elin Einarsson Nastasia J. Freyria Sebastian Gornik Imen Lassadi Arnab Pain	https://www.protocols.io/view/oyster-parasite-perkinsus-marinus-transformation-u-gv9bw96 https://www.protocols.io/view/glass-beads-based-transformation-protocol-for-perk-g36byre https://www.protocols.io/view/fluorescence-activated-cell-sorting-facs-of-perkin-hh2b38e https://www.protocols.io/view/golden-gate-plasmids-used-for-transfection-of-perk-37egrije	Genebank (EF632302, EF632303, KX423758–60)
<i>Oxyrrhis marina</i>	CCMP 1788 CCMP 1795	Patrick Keeling Claudio Slamovits Senjie Lin	Elizabeth C. Cooney Nicholas A. T. Irwin Elisabeth Hehenberger Yoshihisa Hirakawa Brittany Sprecher Lu Wang Huan Zhang	https://www.protocols.io/view/transfection-of-alexa488-labelled-dna-into-oxyrrhi-ha8b2hw https://www.protocols.io/view/calcium-phosphate-transfection-of-oxyrrhis-marina-ha4b2gw https://www.protocols.io/view/electroporation-transformation-of-fitc-dextran-int-3cmgiu6 https://www.protocols.io/view/Dinoflagellate-transformation-e6bbhan https://www.protocols.io/view/co-incubation-protocol-for-transforming-heterotroph-7pmmn https://www.protocols.io/view/electroporation-of-oxyrrhis-marina-vcne2ve https://www.protocols.io/view/co-incubation-protocol-for-transforming-heterotroph-hmzb476	
<i>Hematodinium sp.</i>	Submitted to ATCC collection (in the meantime please contact Waller's lab if interested)	Ross Waller	Sebastian Gornik Ilan Hu Imen Lassadi Arnab Pain	https://www.protocols.io/view/plasmid-used-for-transfection-trials-of-hematodini-4nigvce	

<i>Fugacium (Symbiodinium) kawagutii</i>	CCMP 2468	Senjie Lin	Brittany Sprecher Lu Wang Huan Zhang	https://www.protocols.io/view/dinoflagellate-transformation-7prhmm6	
<i>Alexandrium catenella</i>	CCMP BF-5	Senjie Lin	Brittany Sprecher Lu Wang Huan Zhang	https://www.protocols.io/view/dinoflagellate-transformation-7prhmm6 https://www.protocols.io/view/nucleofector-protocol-for-dinoflagellates-using-lo-7n8hmhw	
<i>Karlodinium veneficum</i>	CCMP 1975	Senjie Lin	Brittany Sprecher Huan Zhang	https://www.protocols.io/view/dinoflagellate-transformation-7prhmm6 https://www.protocols.io/view/nucleofector-protocol-for-dinoflagellates-using-lo-7n8hmhw	
<i>Breviolum (Symbiodinium) sp.</i>	NIES-4271	Jun Minagawa	Yuu Ishii Konomi Kamada Shinichiro Maruyama	https://www.protocols.io/view/electroporation-of-fluorescein-into-the-coral-symb-hdcb22w	https://www.protocols.io/view/dna-construct-for-genetic-transformation-of-the-co-7udhns6/document
<i>Cryptocodinium cohnii</i>	CCMP 316	José Fernández Robledo Ross Waller	Duncan B. Coles Nastasia J. Freyria Paulo A. Garcia Imen Lassadi	https://www.protocols.io/view/transfection-of-cryptocodinium-cohnii-using-label-z26f8he	Probe amplified from EF632302
<i>Amphidinium carterae</i>	CCMP 1314	Christopher Howe	Adrain Barbrook Isabel Nimmo Ellen Nisbet	http://dx.doi.org/10.17504/protocols.io.4r2gv8e https://www.protocols.io/view/biolistic-transformation-of-amphidinium-hnmb5c6	
Discobans (Euglenozoans and Heteroloboseans)					
<i>Bodo saltans</i>	submitted to ATCC collection	Virginia Edgcomb	Miguel A. Chiurillo Roberto Decampo Fatma Gomaa Noelia Lander Zhuhong Li	http://dx.doi.org/10.17504/protocols.io.s5peg5n http://dx.doi.org/10.17504/protocols.io.s5meg46 http://dx.doi.org/10.17504/protocols.io.s5jeg4n http://dx.doi.org/10.17504/protocols.io.sh4eb8w http://dx.doi.org/10.17504/protocols.io.sh6eb9e	http://dx.doi.org/10.17504/protocols.io.7fchjiw GenBank (MN608152)
<i>Diplonema papillatum</i>	ATCC 50162	Julius Lukeš	Drahomíra Faktorová Ambar Kachale Binnypreet Kaur Getraud Burger Matus Valach	https://www.protocols.io/groups/julius-lukes https://dx.doi.org/10.17504/protocols.io.4digs4e	GenBank (MN047315)
<i>Eutreptiella gymnastica</i>	SCCAP K-0333	Vladimir Hampel	Natalia Ewa Janowicz Anna M.G. Novák Vanclová	https://www.protocols.io/view/protocols-for-mrna-electroporation-hh4b38w	

				https://www.protocols.io/view/nucleofection-of-pyramimonas-parkeae-chromera-veli-ibucanw	
				https://www.protocols.io/view/biolistic-transformation-experiment-on-eutreptiell-ibvcn6	
<i>Naegleria gruberi</i>	ATCC 30224	Anastasios Tsaousis	Veronica Freire-Beneitez Eleanna Kazana Jan Pyrih Tobias von der Haar	http://dx.doi.org/10.17504/protocols.io.hnhb5b6 http://dx.doi.org/10.17504/protocols.io.hpub5nw http://dx.doi.org/10.17504/protocols.io.hpvb5n6	http://dx.doi.org/10.17504/protocols.io.7w4hpgw
Opisthokonts					
<i>Pirum gemmata</i>	ATCC PR-280	Elena Casacuberta Iñaki Ruiz-Trillo	Cristina Aresté	http://dx.doi.org/10.17504/protocols.io.z5nf85e	
<i>Sphaeroforma arctica</i>	ATCC PRA-297	Elena Casacuberta Iñaki Ruiz-Trillo	Cristina Aresté Omaya Dudin	http://dx.doi.org/10.17504/protocols.io.z5nf85e http://dx.doi.org/10.17504/protocols.io.z6ef9be	
<i>Abeoforma whisleri</i>	ATCC PRA-279	Elena Casacuberta Iñaki Ruiz-Trillo	Elena Casacuberta Cristina Aresté Sebastián Najle	https://www.protocols.io/view/abeoforma-whisleri-transient-transfection-protocol-zexf3fn http://dx.doi.org/10.17504/protocols.io.zexf3fn	
<i>Salpingoeca rosetta</i>	ATCC PRA-390	Nicole King	David Booth Monika Sigg	http://dx.doi.org/10.17504/protocols.io.h68b9hw	

- American Type Culture Collection (ATCC) (<https://www.atcc.org/>)
- Culture Collection of Marine Phytoplankton (CCMP) now The Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA) (<https://ncma.bigelow.org/cms/index/index/>)
- Kuehnle AgroSystems Inc (KAS) (<http://www.kuehnleagro.com/>)
- Scandinavian Culture Collection of Algae and Protozoa (SCCAP) (<http://www.sccap.dk/dk/soeg/detaljer.asp?Cunr=K-0007>)
- Microbial Culture Collection at the National Institute for Environmental Studies (NIES Collection, Tsukuba, JAPAN) (<https://mcc.nies.go.jp/>)
- Culture collection of the BCCM-DCC (<http://bccm.belspo.be/about-us/bccm-dcg>)
- Culture collection MLML-EBL (<https://www.mlml.calstate.edu/ebi/>)

Reference

Djouani-Tahri, el B., Sanchez, F., Lozano, J. C., Bouget, F. Y. (2011). A phosphate-regulated promoter for fine-tuned and reversible overexpression in *Ostreococcus*: application to circadian clock functional analysis. *PLoS One* **6**: e28471.

Details of protocol for particular species:

¹*Micromonas commoda*: Acclimated mid-exponential *M. commoda* cells grown in L1 medium at 21 °C were spun at 5000 x g for 10 min, the pellet was resuspended in Buffer SF (Lonza) premixed with carrier DNA (pUC19) and plasmid, and 3 x 10⁷ cells were used per reaction. After applying the EW-113 pulse, 100 µl of ice-cold recovery buffer (10 mM HEPES-KOH, pH 7.5; 530 mM sorbitol; 4.7% [w/v] PEG 8000) was added to each well and incubated for 5 min at room temperature. Each reaction was then transferred into 2 ml L1, placed at 21 °C and light was increased stepwise over 72 h.

²*Perkinsus marinus*: In brief, a newly formulated transformation 3R buffer (200 mM Na₂HPO₄; 70 mM NaH₂PO₄; 15 mM KCl; 1.5 mM CaCl₂; 150 mM HEPES-KOH, pH 7.3) was used to reduce the cost of electroporation. 5 x 10⁷ cells were resuspended in 330 µl of fresh ATCC Medium 1886 and were mixed with 5.0 µg of linearized and circular [1:1] plasmid and 300 µl of glass beads (Sigma) in a 1.5 ml tube, vortexed for 30 s at maximum speed, and cells in 500 µl of culture medium were transferred to 6-well plates in a final volume of 3 ml.

³*Bodo saltans*: Square-wave electroporation (Nepa21) was used with a poring pulse of 250V (25 ms) and 5 transfer pulses of 60V (99 ms) in the presence of Cytomix buffer (120 mM KCl; 0.15 mM CaCl₂; 10 mM KH₂PO₄; 2 mM EGTA; 5 mM MgCl₂; 25 mM HEPES-KOH, pH 7.6).